Chulak L. D., Zadorozhny V. G., Chulak Yu. L., Chulak O. T., Tatarina O. V. Ultrasonic extraction of amaranth oils. Journal of Education, Health and Sport. 2018;8(8):1227-1235. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1465021
http://ojs.ukw.edu.pl/index.php/johs/article/view/6203

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part b item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 02.08.2018. Revised: 14.08.2018. Accepted: 31.08.2018.

Ultrasonic extraction of amaranth oils

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Abstract

The isolation of biologically active substances - extraction, is currently the most difficult and time-consuming task that can be solved only in the conditions of large productions with the help of specialized equipment, and practically impossible at home.

As numerous studies show, from the natural raw material of plant and animal origin it is possible to extract practically all known compounds, which are produced by plants [1].

The authors carried out extraction of amaranth in cold pressed olive oil. Since it is close in composition with amaranth oil.

The biological activity of the oil is due to the presence of squalene and tocotrienol, and the percentage of their various fractions possessing antioxidant properties.

Key words: amaranth oil, ultrasonic extraction, squalene

Despite the rapid development of the production of synthetic drugs, most biologically active substances are derived from natural raw materials of plant or animal origin. The share of herbal preparations accounts for 77% of cardiac, 72-75% of expectorant and stomach aids.

The isolation of biologically active substances - extraction, is currently the most difficult and time-consuming task that can be solved only in the conditions of large productions with the help of specialized equipment, and practically impossible at home.

As numerous studies show, from the natural raw material of plant and animal origin it is possible to extract practically all known compounds, which are produced by plants [1].

The ultrasonic method of extracting biologically active substances is economically profitable in industrial production, and the received preparations meet all requirements of pharmacology [2].

When extraction of vegetable raw materials it is usually necessary to use dried materials. Therefore, at the first stage of extraction it is necessary to soak the plant material. Typically, a 5 to 10 hour period is required for soaking.

Ultrasonic fluctuations can significantly reduce soaking time.

Before proceeding with ultrasonic extraction it is necessary to provide the necessary dispersion of the raw material. When using grass plants with a thin, loose plate with soft membranes and a large number of tissues, intercellular spaces, the particle size does not play a significant role and can vary from 2 to 8 mm as a raw material. In the case of hard shells such as amaranth, the size of the particles should be in the range of 0.1-0.5 mm.

After selecting the raw material and its crushing, it is necessary to select the liquid in which the extraction will be carried out. In this case there are no special restrictions on the use of different solvents. If the extractant is not explosive, does not decompose, such an extractant can be used. The best extraction results are obtained with the use of alcohol-water mixtures and vegetable oils [3].

Adding small amounts of surfactants to the extractant (0,1 ... 0,3%) provides an increase in the yield of useful substances.

When performing ultrasonic extraction, it is necessary to ensure the access of the extractant to each particle and to exert an ultrasound effect on each particle. This can be achieved either by intensive mixing or reduction of the ratio: the raw material is the extractant.

With an increase in the time of exposure, the amount of biologically active substances increases proportionally. But an increase can not occur infinitely, because the raw material is depleted. Full depletion of raw materials with a particle size of 0.5 mm and the effect of ultrasound occurs within 15 minutes. With a particle size of 1 mm, complete exhaustion occurs in 60 minutes. processing 2 hours is required for the complete exhaustion of raw materials with particle size of 2 mm. With a particle size of 8-10 mm for two hours of processing less than 55% of biologically active substances is released. Thus, preliminary crushing of raw materials to particle size less than 0.5 mm provides under optimum conditions the complete depletion of raw materials for 10-15 minutes of ultrasonic treatment.

With the increase in the temperature of the extractant, intensive formation of gas bubbles begins at the boundaries of the section and the intensity of the transmission of ultrasonic energy decreases. Therefore, the maximum yield of biologically active substances occurs at a temperature of 30-60 degrees.

When extracting, it is necessary to take into account the increase in the temperature of the extractant by absorbing ultrasonic energy and to ensure that the temperature of the extract does not exceed the permissible values. High temperatures lead to destruction, which, in turn, leads to the destruction of the conformation of macromolecules and reduces the use of such extracts to zero [4, 5].

Extraction of amaranth was carried out in cold pressed olive oil. Since it is close in composition to amaranth oil.

Experimental installation is presented in Fig. 1. The amaranth grain was pre-grounded to a maximum of 0.2 mm in size to increase the area of contact with olive oil.

Fig.1 Laboratory Ultrasonic Plant for Amaranth Oil Extraction

The ratio is 50% to 50% by volume. The resulting mixture was placed in a container which was exposed to ultrasound at a frequency of 40 kHz. Using a higher frequency can lead to the destruction of components of extracted oil. Specific power per unit area of the emitter varied from 3 to 5 W per square centimeter. For a uniform distribution of amaranth in volume

during extraction a glass stirrer with an electric drive was used. The temperature during extraction was measured using a thermocouple and a thermoswitch, which stopped the extraction process if the temperature exceeded 40 degrees. Extraction time 1, 2 and 3 hours, respectively.

Fig.2 Chromatomas-spectrometer Agilent-6890-N/5975 InertGC/msSistem

To determine the composition of triglycerides of higher acids, the following test preparation was carried out. One gram of samples of liquids weighed on analytical scales with an accuracy of 0.1 mg was dissolved in an exact amount of hexane, selected with a pipette of 10 ml after thorough mixing, 2 ml of solution, selected by pipette of grade 2 accuracy, transferred to a box with a hardened lid and added 1 ml of sodium methoxide.

After stirring in 1 hour, 5 ml of distilled water was added and a layer of hexanes was collected which was examined by chromatographic method using a mass detector. Installed Identification of substances was carried out using libraries of mass spectra. Quantitative content of higher acids was determined by the method of external stanardt. To do this, individual qualification materials were used at least HH.

In order to construct a calibration graph, acid methylates with precise concentrations were prepared analogously. For the determination of quantitative content, the peak areas of individual substances were compared with the values of the areas of the peaks of the same substances with known concentrations applied on the calibration graphs. The error of the quantitative analysis did not exceed 0.2% at the value of the confidence interval of 0.95. To determine the concentration of squalene, the test was to prepare the previous amalgamation of triglycerides with the extraction of unlabeled substances. For this purpose, 10 grams (exact

weighting) of the sample oil was poured into a round bottom flask of 100 ml capacity, which was added 50 ml of a 20% solution of sodium hydroxide, connected the refluxer and heated in a water bath for 2 hours.

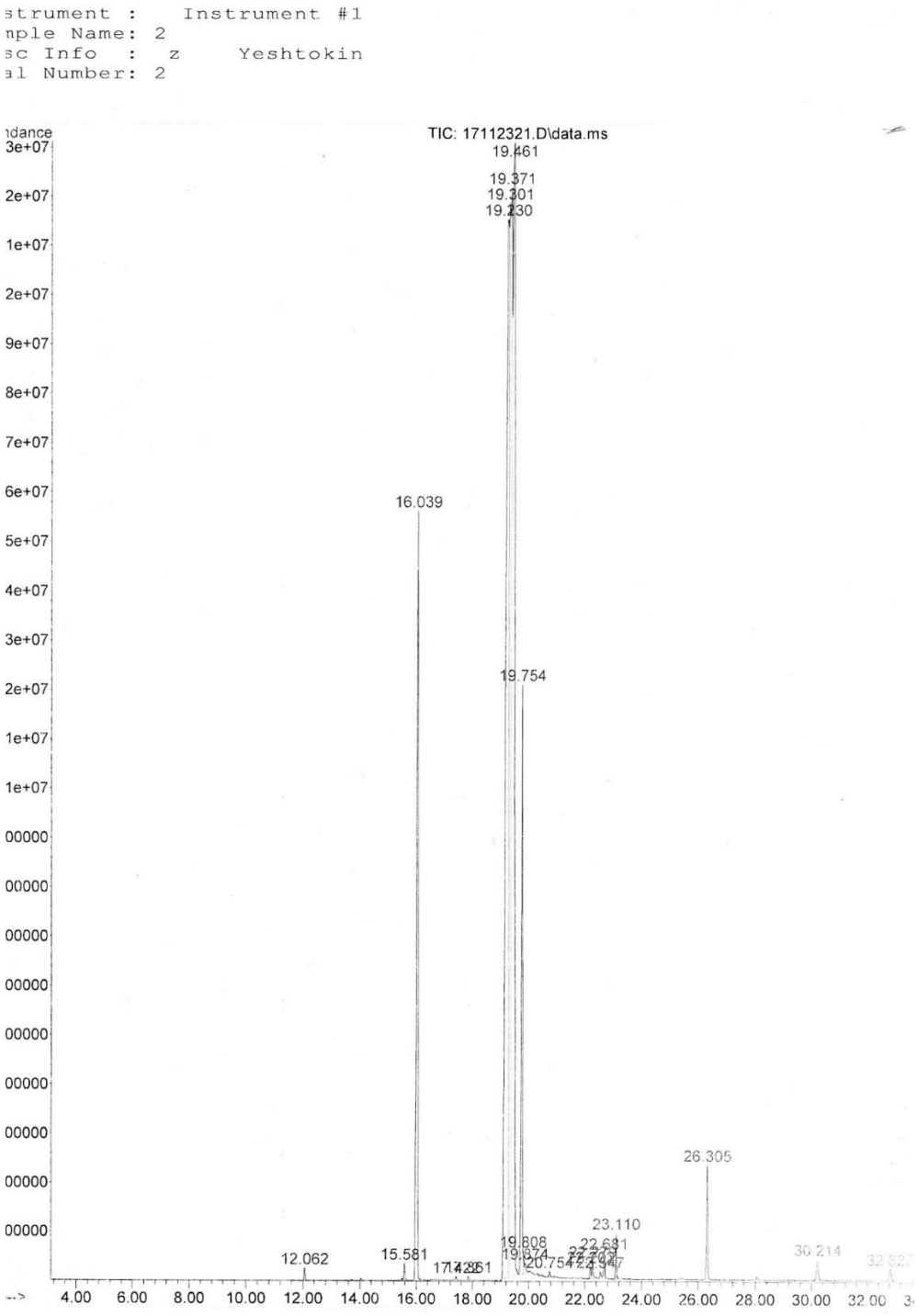

Fig.3. Chromatogram of amaranth extract P-5 W / cm2. Extraction time-3 hours

After washing, three times the "OSM for chromatography" was added to the flask, 10 ml, which was separated from the aqueous layer by a separating funnel. The combined hexanes extract was dried over anhydrous sodium sulfate and evaporated to a 50 ml volumetric flask. The obtained precision solution was investigated by chromatographic method using the chromatomas-spectrometer Agilent-6890 N / 5975 - InertGC / MC System with a capillary column - HP-5MS, 30 m in length, 0.25 mm in diameter, 0.25 μm in phase, 0.25 μm in gas, gel carrier , ionization - electron impact, ionization energy - 70 eV, ion source temperature - T-230^0 C / temperature of quadropolis - T-150^0 C

The resulting chromatograms were processed using the NISTv2.0 mass spectrometry library. As values for constructing a gauge graph, the values of squalene peak areas with known concentration were determined. To determine the quantitative content of squalene, the resulting area of the sample was compared with the value of squalene peak areas with known concentration applied on the calibration graph. The error of the quantitative analysis does not exceed 0.1% at the confidence interval value of 0.95.

The results of mass-chromatogram processing are presented in Table 1.

Table 1. Impact of ultrasound power - radiator and extraction time to the composition of the amaranth oil obtained (t <40º C, frequency 40 kHz)

storage acids	3 W			5 W		
	1 hour	2 hours	3 hours	1 hour	2 hours	3 hours
	%	%	%	%	%	%
meristan acid	0,1	0,15	0,4	0,2	0,3	0,5
palmitic acid	5,2	6,0	7,1	6,6	8,5	9,1
oleinic acid	58	58	60	60	66	68
linoleic acid	5,1	5,6	6,1	8,7	8,9	9,1
stearic acid	2,1	3,2	4,0	3,5	4,0	4,9
arachidon acid	0,1	0,1	0,2	0,1	0,2	0,4
	0,15	0,22	0,5	0,8	0,9	1,1
Squalene	1,1	1,8	2,6	3,1	3,5	6,6

As can be seen from Table 1, the highest concentration of squalene is obtained by extraction of the amaranth for 3 hours and the power of ultrasound 5 W / cm2. Increasing the power of the emitter leads to an increase in the temperature of the extractor and the destruction of components of amaranth oil.

The biological activity of the oil is due not only to the presence of squalene and tocotrienol as such, but also to the percentage of their various fractions, possessing antioxidant properties, and fractions that have biological activity.

The quality of vegetable oils is evaluated according to the organoleptic and physico-chemical parameters. And the importance of these indicators is largely determined by the purpose of the oil.

To organoleptic parameters include taste, smell, color and transparency. The taste and smell of vegetable oils depend on the type and quality of the raw material being processed, on the mode of production (pressing or extraction) and on the technological modes of equipment operation. Cheese benign vegetable oils have a specific taste and smell for this type of oil. In the oil, foreign flavors and smells, bitterness and shading are not allowed. By taste and smell you can set the type of oil, to a certain extent benign, as well as the presence of various impurities. After refinement, the taste and smell of the oil become less pronounced. The color of the raw vegetable oils is rather specific, but it depends heavily on the method of extracting the oil (so, the extractive oils have a more intense color than the press), as well as from the conditions of their storage. It is known that under the action of oxygen in air, ultraviolet and gamma-radiation, the oil gradually discolorates. Transparency - muta or suspended particles that are noticeable by the naked eye, which worsen the merchandising of the oil, reduce the variety. Physico-chemical indicators include: moisture content and volatile substances; acidic, colored, iodine; the content is not fatty admixtures; content of phosphorus-containing substances; outbreak temperature One of the main characteristics of the quality of oil, its suitability for food purposes - an acid number. It describes the content of free fatty acids in the oil, the presence of which is mainly due to the process of splitting the molecules of glycerides in the absence of regimes of storage of oilseeds, violation of the technological process of production, as well as the incompleteness of the formation of molecules triacylglycerols of glyceride molecules in the absence of regimes of storage of oilseeds, violation the technological process of production, as well as the incompleteness of the processes of formation of triacylglycerol molecules due to the unfavorable weather conditions in growing plants. The accumulation of free fatty acids in oil indicates a deterioration in its quality. For edible oils, the acid number should not exceed 4.0 mg KOH / g. The color of the oil shows the intensity of its color, that is, the presence of carotenoids. In refined oil, the color number is 10-12, in unrefined it ranges from 15 to 35. In refined oil, there are no phospholipids, which determines its low biological value. Hydrated oil also loses on this indicator unrefined.

Also, from the refined and hydrated oil completely removed non-fat (non-sticking) impurities, therefore they do not contain sediment and sediment. The content of pesticides, toxic elements, mycotoxins, and radionuclides in the oil must not exceed the permissible limits established by medical-biological requirements and sanitary norms of quality for food raw materials and food products.

The results of the physico-chemical studies of the oil we obtained are shown in Tables 2 and 3.

Таблиця 2.

Name indicators	Amaranth oil	
	5 W / cm^2, τ = 3 years. Kharkiv-therapeutic	literature / 6 /
Density, ρ^{20}, g / dm^2	0,8907	0,8872
Refractive index	1,4680	1,4645
Acid number Mg KOH / g	[+] 5,07	5,05
Number of sapling Mg KOH / g	184,6	185,73
Iodine number I_2/100 *g*	101,2	102,03
Peroxide number mmol / kg	1,28	1,27
Mass fraction of ash, g / 100 g of oil	0,30	0,31

Table 3.

Indicator to be determined	Permissible content	Actual content	Method of workout
TOXIC ELEMENTS (mg / kg):			
Lead	0,1	0,015	GOST 31078-96
	0,05	0,002	31078-96
Arsenic	0,1	0,000	26930-86
Mercury	0,05	0,001	МУ 5178-90
M / B INDICATORS:			
Pathogenic Salmonella		Not detected	GOST 30519-97

Conclusions. Our data allow us to assert that the extraction of the amaranth proposed by us with the use of ultrasound effects significantly reduces the extraction time and reduces energy consumption. According to its composition and chemical parameters, the extract obtained does not differ from amaranth oil cold press. The content of squalene reaches 6.6%.

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